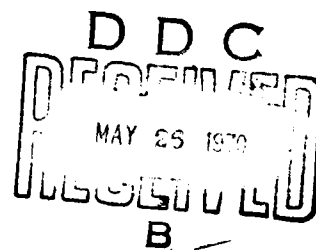


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INFLUENCE OF TEMPERATURE ON THE EFFECTS OF ENTEROTOXIN AND ENDOTOXIN IN MICE

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March 1970

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FOREWORD

This research was accomplished in the Microbiology-Immunology Branch, under task No. 775309, between January and May 1969. The paper was submitted for publication on 12 January 1970.

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences-National Research Council.

For this research, the B-type enterotoxin (SEB) was supplied by Dr. E. J. Schantz, Ft. Detrick, Frederick, Md.; and the lipopolysaccharide endotoxin (LPS) was secured from the Difco Laboratories, Detroit, Mich.

The author expresses his appreciation to Alton J. Rahe, Biometrics Division, for the statistical analyses.

This report has been reviewed and is approved.


JOSEPH M. QUASHNOCK
Colonel, USAF, MC
Commander

ABSTRACT

For optimum health, the host must control the proliferation of endogenous bacteria and neutralize harmful toxins. Staphylococcal enterotoxin B (SEB) produced by gram-positive bacteria and lipopolysaccharide endotoxin (LPS) produced by certain gram-negative bacteria can exert synergistic effects when injected into homothermic animals (mice). In both acclimatized and nonacclimatized animals, exposure to temperature extremes of 4° C. or 35° C.—except in one instance—either hastens or increases death in the presence of SEB and LPS when compared to room temperature (24° C.). This research determines experimentally that ambient temperature is a meaningful variable in the resistance or susceptibility of a mammal to certain common bacterial toxins.

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I. INTRODUCTION

Staphylococcal enterotoxin (SE) and lipopolysaccharide endotoxin (LPS), separately and in synergistic combination, are the subject of this research. In man, SE is a common cause of food poisoning (1); and LPS, produced by certain gram-negative bacteria, causes fever. The bacteria that produce these toxins are common genera which may inhabit man and other animals. In these hosts, effective concentrations of the toxins may occur simultaneously.

No observable effect occurs when SE is injected into mice, and LPS is only slightly incapacitating; but the combination, when given in sequence at certain concentrations, is highly lethal.

II. MATERIALS AND METHODS

Toxins

The SE was a lyophilized extract of *Staphylococcus* spp. Fractionation of this extract resulted in a B-type enterotoxin, generally referred to as SEB (2). The LPS endotoxin was a commercial preparation of lipopolysaccharide obtained from *Salmonella enteritidis*. The animals were administered, intraperitoneally (I.P.): 12 μ g. of SEB in 0.1 ml. of saline; and, approximately 4 hours later, 150 μ g. of LPS in 0.1 ml. of saline. To minimize circadian variability, the SEB injections were given between 8:00 and 9:00 a.m. and the LPS injections between 12:00 and 1:00 p.m. (3).

Animals and housing

Male mice, of the Swiss-Webster strain, were used in this research. They ranged in

weight from 20 to 26 gm. The animals were housed in groups of two in plastic cages (28 cm. x 18 cm. x 13 cm.) having perforated metal lids and pellet troughs. Small groups of mice were preferred in order to increase the liberation of body heat at the higher temperature and to decrease any effects of huddling at the lower temperature. Food and water were provided ad libitum. Sawdust bedding was kept to a minimum to limit the burrowing tendency expected at the lower temperature.

Environmental conditions

Three temperatures were selected for study: 35° C., 24° C., and 4° C. The 24° C. environment was the normal laboratory condition and served as a control. (Acclimatization to 24° C., when stated in figure legends, signifies that the mice were not moved from any other temperature to 24° C.) The high temperature was maintained by placing the cages in an incubator room; 4° C. was the temperature of the cold room. Temperature variation was approximately $\pm 2^\circ$ C. The conditions are summarized in table I.

III. RESULTS

In figure 1 are shown the lethality curves for mice exposed to 35° C., 24° C., and 4° C. under acclimatized (48 hours) and nonacclimatized conditions. These mice were injected with saline and, 4 hours later, with LPS. Evidently the trauma of sudden ambient temperature change increases the susceptibility of nonacclimatized mice to LPS endotoxin (fig. 1: exp. 1 vs. exps. 2, 3) at both 4° C. and 35° C.

When the animals were acclimatized for 48 hours before being injected with saline and

TABLE I
Ambient conditions and injections used on mice to explore influence on effects of SEB and LPS

Exp. No.	Temp. (deg. C.)	Acclimatized	SEB	LPS	Remarks
1	24	No	No	Yes	Saline for SEB
2*	35				
3*	4				
4	4				
5	35	48 hr.	Yes	Yes	Control
6	24				
7	4				
8	35				
9	4	48 hr.	Yes	Yes	—
10	35				
11*	35				
12*	4				
13*	35	No	Yes	No	Saline for LPS
14*	4				
13*	35	No	No	No	Saline-temp. control
14*	4				

*Only 10 mice per experiment. Each of the other experiments had 20 mice.

later with LPS, only the 35° C. temperature (exp. 5) increased their susceptibility to a statistically significant probability level.

Experiments 11 and 12, in which SEB is used before saline, were conducted as additional controls. (These experiments are not included in figure 2 because no deaths occurred at 4° C. or 35° C.)

As shown in figure 2, both the temperature-acclimatized (exp. 9, 10) and the nonacclimatized (exp. 7, 8) mice die at a faster rate at 4° C. (exp. 7, 9) and 35° C. (exp. 8, 10) than at 24° C. (exp. 6). Moreover, in the nonacclimatized group, at 35° C. (exp. 8), the increased deaths at 3.5 hours were statistically

significant compared to those at 24° C. (exp. 6) and at 4° C. (exp. 7).

IV. DISCUSSION

The mechanism and effects of endotoxin from gram-negative bacteria have stimulated considerable research for two decades (4-6). In addition to vascular effects, other alterations (e.g., pyrexia, leukopenia, leukocytosis, hyper- and hypoglycemia, changed resistance to infections, and tumor necrotizing capacity) have been associated with LPS (7). In the extreme, these symptoms lead to death of the host.

According to one report at least, oxygen tensions and cell anoxia resulting from metabolic events are responsible for endotoxin

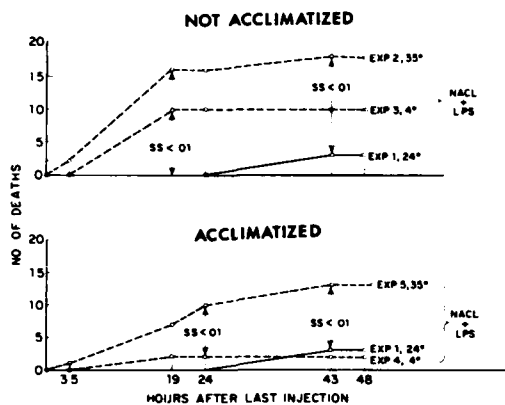


FIGURE 1

Total deaths vs. hours for mice after last injections of 0.1 ml. of saline (I.P.) and, 4 hours later, of 150 μ g./0.1 ml. of LPS (I.P.). [Before injection, the acclimatized groups were held 48 hours at the temperatures shown. Statistically significant (SS) differences and probability levels are indicated. Experiments 2 and 3 had 10 animals per group. The number of deaths was doubled to facilitate graphing.]

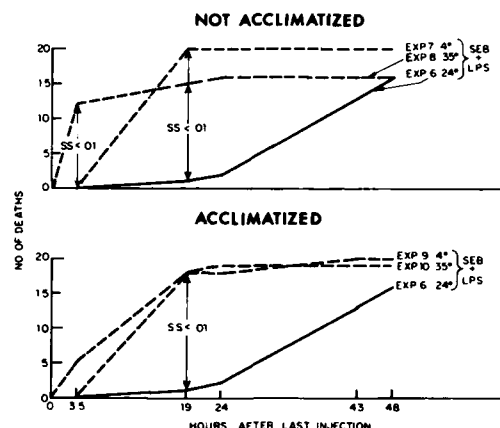


FIGURE 2

Total deaths vs. hours for mice after last injections of 12 μ g./0.1 ml. of SEB (I.P.) and, 4 hours later, of 150 μ g./0.1 ml. of LPS (I.P.). [Before injection, the acclimatized groups were held 48 hours at the temperatures shown. Statistically significant (SS) differences and probability levels are indicated.]

shock. Glycorticoids from the adrenal cortex seem to be effective in protecting some animals from endotoxin (8). Some evidence indicates that adrenal hypertrophy occurs in hamsters kept at 5° C. (9), and it is reasonable to suspect that the same occurs in mice. Such hypertrophy would increase the corticosteroids and

thus increase resistance. As for saline and LPS only, ambient temperature affects the rate of death and eventual overall mortality in both acclimatized and nonacclimatized animals, except in one instance (fig. 1): acclimatized mice seem to combat toxin effects better at 4° C. than at 35° C.

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